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Monoclonal Antibody Specific for *Listeria monocytogenes*, *Listeria innocua*, and *Listeria welshimeri*

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Eight hundred fifty-nine murine hybridomas were produced from eight fusions, and 27 were characterized for secretion of antibodies reactive to *Listeria monocytogenes*. One monoclonal antibody (MAb), P5C9, reacted with all test strains of *L. monocytogenes* (31 of 31), *L. innocua* (3 of 3), and *L. welshimeri* (1 of 1) but not with any strains of the other four *Listeria* species or with any of 22 gram-positive or 11 gram-negative species of bacteria when tested in microtiter and dot blot enzyme immunoassays. Of the other 26 antibodies, 20 reacted with either *L. monocytogenes* Scott A or V7 and with some or all of the other six *Listeria* species but also cross-reacted with some or all of the non-*Listeria* bacteria tested. MAb P5C9 is of the immunoglobulin G1 murine subclass. In Western blot (immunoblot) analyses, this MAb reacted with a single antigen with a molecular weight of 18,500, and it is shared in common with all three reactive species, *L. monocytogenes*, *L. innocua*, and *L. welshimeri*. This antigen was extracted with detergent and appeared to be cell bound.

Foodborne listeriosis is a problem of major concern to the public health community and food processors. Several foodborne cases have occurred in North America (9, 20, 35), prompting the U.S. Food and Drug Administration (USFDA) and the U.S. Department of Agriculture (USDA) to establish a zero tolerance for *Listeria* species in 25-g samples of cooked, ready-to-eat foods, including meat and poultry products.

Many culture methods and media for *Listeria* species have been devised and reported which appear to be reliable but are time-consuming (7, 11, 21, 23, 38). The use of enrichment culture protocols coupled with rapid detection systems (immunoassay and nucleic acid probes) are perhaps the most efficient methods available (5, 16, 22).

The immunoassay systems commercially available in the United States for *Listeria* detection are specific to the genus level only but fulfill the current regulatory needs of detecting any of the seven recognized *Listeria* species. The availability of more rapid immunoassay methods to specifically detect *Listeria monocytogenes*, the main species reported to be pathogenic to humans (10, 24), would be very useful in tracing the distribution of this pathogen in environmental, food, and clinical specimens.

In this report, we describe the production and screening of 27 hybridoma antibodies and the characterization of one monoclonal antibody (MAb), P5C9, which showed reactivity toward *L. monocytogenes*, *L. innocua*, and *L. welshimeri* but not toward the other four recognized *Listeria* species. MAb P5C9 showed no cross-reactivity with a variety of other starter cultures and other non-*Listeria* bacteria commonly associated with foods or isolated from food-processing environments by the selective culture methods for *Listeria* currently recommended by the USFDA and the USDA Food Safety and Inspection Service. Partial characterization of the cellular antigen which binds with P5C9 is also presented.

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MATERIALS AND METHODS

Organisms. Bacterial cultures were maintained at −20°C as washed cell suspensions in 100% glycerol, glycerol-overlaid agar slants, or broth cultures in 10% nonfat dry milk solids. The *Listeria* species and strains used are detailed in Table 1. The non-*Listeria* species used were *Jonesia denitrificans* ATCC 14870, *Bacillus cereus* UADFS, *B. subtilis* UADFS 54, *Lactobacillus delbrueckii* UADFS 43, *L. casei* NRRL B1445, *L. plantarum* ATCC 8014, *L. bulgaricus* UADFS, *Leuconostoc mesenteroides* UADFS 10, *Micrococcus varians* UADFS 69, *M. luteus* UADFS 36, *Streptococcus cremoris* UADFS 56, *S. lactis* C-2, *S. faecalis* ATCC 344, *S. thermophilus* UADFS, *S. pyogenes* ATCC 19615, *Erysipelothrix rhusiopathiae* ATCC 805, *Corynebacterium bovis* ATCC 7715, *Brochothrix thermosphacta* ATCC 11509, *Pediococcus cereviseae* UADFS 67, *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Proteus mirabilis* UADFS, *P. vulgaris* ATCC 14028, *Salmonella typhimurium* UADFS 46 and ATCC 14028, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Enterobacter cloacae* ATCC 23355, *Serratia marcescens* ATCC 8100, *Citrobacter freundii* ATCC 3624, *Pseudomonas aeruginosa* ATCC 19142, *P. fluorescens* UADFS 58, and *P. fragi* ATCC 4973. All *Lactobacillus* and *Pediococcus* cultures were grown in Lactobacillus MRS Broth (Oxoid Co., Columbia, Md.). The remaining cultures were grown in tryptic soy broth–0.5% yeast extract (TSBYE broth or agar; Difco Laboratories, Detroit, Mich.). All *Listeria* strains were cultured at 37°C unless otherwise noted.

Immunogen preparation. *L. monocytogenes* V7 (serotype 1/2a) and Scott A (serotype 4b) and *L. innocua* ATCC 33090 were used to prepare separate immunogens. Cells were grown as lawns on TSBYE agar in 150-cm^2 Roux flasks for 36 h at 37°C to prevent formation of flagellar proteins. Cells were harvested with 0.1 M sodium phosphate buffer (PB), pH 7.2, centrifuged at 4,400 × g at 6°C, and washed twice in

TABLE 1. Bacterial strains and sources

Species and strain	Origin	Source[a]
L. monocytogenes		
Scott A	Human	USFDA
V7	Raw milk	USFDA
Brie-1	Cheese	USFDA
V37CE	Milk	USFDA
Murray B	Human	USFDA
V37	Milk	USFDA
ATCC 15313	Rabbit	ATCC
ATCC 35152	NK[b]	ATCC
F2379	Cheese	USCDC
F1057	Milk	USCDC
LCDC 81-861	Cabbage	University of Georgia[c]
F5069	Egg	North Carolina State University[d]
CAP-Unk.	Human	Arkansas State Health Department[e]
171	Human	Arkansas State Health Department
USDA-RB	NK	USDA-ARS
F1109	Milk	USDA-ARS
S433	Poultry	UADFS
S434	Poultry	UADFS
S435	Poultry	UADFS
S436	Poultry	UADFS
S437	Poultry	UADFS
1-Post5	Environmental	UADFS
3-Pre6-4	Environmental	UADFS
3-Pre6-7	Environmental	UADFS
3-Pre7-1	Environmental	UADFS
3-Pre8-1	Environmental	UADFS
3-Pre10-4	Environmental	UADFS
3-Post6-1	Environmental	UADFS
4-Pre6-4	Environmental	UADFS
4-Pre8-4	Environmental	UADFS
4-Pre12-4	Environmental	UADFS
4-Post6-1	Environmental	UADFS
4-Post8-1	Environmental	UADFS
T83	Rabbitry	UADFS
T66	Rabbitry	UADFS
T47	Rabbitry	UADFS
T20	Rabbitry	UADFS
L. innocua		
ATCC 33090	Cow brain	ATCC
C194	NK	UADFS
L. welshimeri ATCC 35897	Vegetation	ATCC
L. seeligeri		
SE-31	NK	UADFS
MC1412	NK	UADFS
ATCC 19120	Feces	ATCC
L. murrayi ATCC 25401	Vegetation	ATCC
L. ivanovii		
ATCC 19119	Sheep	ATCC
KC1714	NK	USCDC

[a] ATCC, American Type Culture Collection (Rockville, Md.); USCDC, U.S. Centers for Disease Control (Atlanta, Ga.); ARS, Agricultural Research Service (Athens, Ga.); UADFS, University of Arkansas Department of Food Science Culture Collection (Fayetteville, Ark.).
[b] NK, Not known.
[c] Department of Food Science (Griffin).
[d] Department of Food Science (Raleigh).
[e] Little Rock.

PB before being resuspended and kept at 4°C for further processing.

Immunogens for mouse injection were prepared by three different methods. In method 1 (whole heat-killed cells), the cell concentration was adjusted to 3×10^{10}/ml of PB by McFarland turbidity standard curve readings of A_{650} and heated in screw-cap tubes for 1 h at 80°C in a water bath.

In method 2 (cell wall preparation), cells adjusted to the above-described concentration were heated at 100°C for 10 min in flowing steam. To 30 ml of the heated suspension, we added 33 g of 0.12- to 0.18-mm-diameter acid-cleaned glass Ballatini beads in a 90-ml Braun MSK homogenizer flask. The flask volume was brought to two-thirds full with PB. Cells were broken by two 3-min treatments at 2,000 rpm in a Braun MSK cell homogenizer cooled by a stream of CO_2 through the cooling port. Cell walls and wall fragments were harvested and purified by differential centrifugation at 6°C as follows. The broken-cell suspension was centrifuged at 1,940 $\times$ *g* for 10 min, and the supernatant was collected. The supernatant was centrifuged at 23,700 $\times$ *g* for 10 min and decanted, and the pellet was suspended in 10 ml of PB. This step was repeated twice. The resulting pellet was passed successively through 16- and 23-gauge hypodermic needles to resuspend it in the 10 ml of PB and centrifuged at 1,940 $\times$ *g* for 10 min, and the pellet was discarded. The resulting supernatant was centrifuged at 23,700 $\times$ *g* for 10 min, and the supernatant was monitored for UV-absorbing material (A_{260} and A_{280}). This step was repeated until no UV-absorbing material was detected in the supernatant. Wet mounts of the cell wall preparations viewed under phase-contrast microscopy were examined at each stage of processing until no intact cells were viewed in 10 fields at $\times$1,000 magnification. Cell wall fragments were stored at −70°C until used.

In method 3 (Formalin-killed whole cells), cells at the above-described concentration were pelleted by centrifugation. The pellet was suspended in an original volume of PB containing 0.3% formaldehyde and kept at room temperature for 14 h. The cells were then washed three times in PB and suspended in PB. Portions of the suspension were streaked on TSBYE agar plates to confirm that no viable cells persisted. These preparations were stored at −70°C until used.

At least 3 weeks before fusion, individual BALB/c mice were immunized by intraperitoneal injection of 100 μl (300 μg [dry weight]) of diluted immunogen preparations with or without alum precipitation (13) or poly(A)-poly(U) as an adjuvant. At 5 days before fusion, these mice were boosted daily with the same amount of homologous immunogen, allowing 1 day of rest before the fusion was performed.

Hybridoma production. Cultures of murine myeloma cell lines P3X63-Ag8.653 (P3X) and P3/Ns1/1-Ag4-1 (NS-1) were maintained in Iscove modified Dulbecco medium (Sigma Chemical Co., St. Louis, Mo.) with 10% (vol/vol) fetal bovine serum (HyClone Laboratories, Logan, Utah) in 75-cm^2 tissue culture flasks at 37°C in a 7% CO_2 atmosphere. Myeloma cell lines were maintained in the logarithmic growth phase for at least 5 days before fusion. Spleen cells harvested from immunized and boosted mice were fused with either NS-1 or P3X myeloma cells at a ratio of 1:1 by the basic procedure of Kohler and Milstein (17) and distributed among five 96-well plates containing 200 μl of Iscove medium per well with 10% fetal bovine serum (I10F) containing the selective compounds hypoxanthine, aminopterin, and thymidine. The I10F-hypoxanthine-aminopterin-thymidine medium was preconditioned by a feeder layer of approximately 10^3 BALB/c mouse peritoneal lavage cells per well added 24 h before fusion.

About 2×10^5 fusion preparation cells were dispensed into each well. Plates were allowed to incubate for 11 to 14 days, at which time the supernatants from wells showing growth as two-thirds confluence in microscopic examinations were assayed for production of anti-*Listeria* antibody as described below. Selected hybrids (P5C9, 5D10, 5D10F3, 5D10C1, and 8A8) which were positive were selected for subsequent propagation and cloning, followed by rescreening of clones (Table 2). Cloning was performed by limiting dilution in Iscove medium containing hypoxanthine and thymidine.

Screening EIA for anti-*Listeria* antibody. For enzyme immunoassay (EIA), Immulon 1 polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) were coated with cellular antigens (prepared as described above) suspended in 0.05 M carbonate buffer, pH 9.6, to give about 10^8 cells per well. Plates were incubated at room temperature for 16 h on a rotary table at about 50 rpm. Coated plates emptied by inversion were blocked with 100 μl of blocking solution (containing 1% [wt/vol] bovine serum albumin [Sigma], 75 mM glycine in phosphate-buffered saline [PB plus 0.80% NaCl; pH 7.2] [PBS], and 0.01% sodium azide) per well for 2 h at room temperature. Coated and blocked plates were washed once with a standard wash solution of PBS plus 0.5% Tween 20 (PBS-T). Fifty microliters of hybrid cell culture supernatant was added to each well of the plate along with an additional 50 μl of PBS-T. The plates were incubated for 1 h at room temperature on a rotary table, washed four times with PBS-T, and incubated with 100 μl of goat anti-mouse immunoglobulin G (IgG)-horseradish peroxidase conjugate (Sigma catalog no. A-5278) diluted 1:1,000 in PBS-T for 1 h at room temperature with rotary agitation. The plates were then washed four times with PBS-T, and 100 μl of the substrate (*o*-phenylenediamine dihydrochloride [1 mg/ml] in 0.1 M citrate buffer [pH 4.5] containing 0.39 mM H_2O_2) was added per well and allowed to incubate for 15 min at room temperature. The reaction was stopped by addition of 100 μl of 4 N sulfuric acid per well, and the A_{490} was read on a Dynatech MR-600 microplate reader.

Immunoglobulin isotyping and quantitation. The isotype of MAb P5C9 produced in cell culture was determined by agarose gel immunoelectrophoresis and reaction of the electrophoresed sample containing MAb P5C9 against goat anti-mouse IgG subclass 1, 2a, 2b, and 3 antisera. IgG was quantitated by a radial immunodiffusion assay of samples against goat anti-mouse IgG serum. A standard curve based on precipitin zone diameters formed with known amounts of mouse IgG was constructed.

IgG purification from P5C9 hybridoma ascites fluid. P5C9 ascites fluid prepared by using IRCF-1 mice (2) was precipitated with 20% sodium sulfate and resolubilized in 0.2 M sodium phosphate buffer, pH 8.0. After exhaustive dialysis, the protein concentration was adjusted to 8 to 10 mg/ml and 500 mg was loaded onto a preequilibrated column (2.5 by 24 cm) of DEAE–Sepharose CL-6B and eluted by a stepwise gradient of NaCl in 0.025 M Tris hydrochloride, pH 8.0. The protein-containing fractions eluted with 0.11 M NaCl were pooled and assayed for IgG content as described above. A single precipitin band was obtained upon immunoelectrophoresis and subsequent reaction against goat anti-mouse IgG and goat anti-mouse whole serum.

Preparation of rabbit anti-*Listeria* polyclonal antibody. The immunogens described above were also injected into adult New Zealand White or Californian rabbits to produce anti-*Listeria* polyclonal antiserum by the method of Seeliger and Hohne (36). Rabbits were immunized by three weekly intravenous injections of 100 μg (dry weight) with one of six antigens (*L. monocytogenes* Scott A or V7 antigens prepared by methods 1 to 3). Each rabbit was boosted with subcutaneous injections of the homologous immunogens mixed with incomplete Freund adjuvant (Pel-Freez Biologicals Co., Rogers, Ark.). Test bleed serum was assayed for the presence of anti-*Listeria* antibody activity by using the screening EIA described above. Rabbit IgG was purified by precipitation and DEAE ion-exchange chromatography as described earlier and shown to be reactive against strains representing all of the *Listeria* species, as well as many other test bacteria.

Dot blot immunoassays. Cells were diluted in Tris-buffered saline (TBS; 20 mM Tris, 500 mM NaCl [pH 7.5]), and 500-μl amounts were loaded per well and then a vacuum was applied for 30 min at room temperature to immobilize the cellular antigens onto the membrane in a dot vacuum manifold. The membrane was then removed and blocked with 3% (wt/vol) gelatin in TBS for 2 h at room temperature on a rotary table. The blot was then rinsed in one change of TBS–0.05% Tween 20 (TBS-T) and incubated in TBS containing MAb P5C9 (40 μg of IgG per ml) for 1 h at room temperature with slow rotary agitation. The blot was washed by decanting the overlying solution, adding TBS-T, agitating the mixture for 5 min, decanting it, and then washing the blot twice more with TBS-T. This procedure was used for all of the subsequent blot-washing steps.

The washed membranes were then incubated in TBS containing goat anti-mouse IgG-horseradish peroxidase (Sigma catalog no. A5278; diluted 1:600 in TBS) for 1 h at room temperature with slow rotary agitation. The membrane was washed three times in TBS-T, substrate solution (consisting of TBS [28.2 ml], 4-chloronaphthol [3 mg/ml in 1.8 ml of methanol], and H_2O_2 [12 μl]) was added for a final volume of 2.5 ml/cm^2, and the membrane was incubated in the dark for 30 min to 1 h. Color development was stopped by rinsing the membrane in several changes of deionized water.

Western blot (immunoblot) analysis with MAb P5C9 as the immunoprobe. Strains of *Listeria* species were grown in TSBYE medium at 37°C for 36 h. The cells were pelleted, decanted, suspended in 1/10 volumes of PBS, and placed in a boiling water bath for 15 min. The preparations were preserved with 0.02% sodium azide and stored at 5°C. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) of these suspensions was performed by the method of Laemmli (18). Samples were run in a discontinuous minigel system (Mighty-Small II apparatus; Hoefer Scientific Co., San Francisco, Calif.) with a 4% acrylamide stacking gel and a 12% acrylamide separating gel. For gradient gel electrophoresis, the separating gel acrylamide concentration ranged from 5 to 15%. Samples were mixed 1:1 with sample buffer and heated in a boiling water bath for 3.5 min before each well was loaded with 15 μl of a suspension containing about 2×10^8 *Listeria* cells. Gels were run at constant currents of 13 and 18 mA per gel for stacking and separating gels, respectively. Following electrophoresis, portions of the gels were silver stained (Bio-Rad Laboratories silver stain kit) or stained with Coomassie blue. The remaining portions of the gel were transferred to Immobilon P membranes (Millipore Corp., Bedford, Mass.) by means of a semidry blotting apparatus (ABN Polyblot; American Bionetics, Hayward, Calif.). Portions of the blot were stained with amido black. The remaining portion was blocked and probed with MAb P5C9 as described above for dot blots. The identifying conjugate was either a peroxidase

TABLE 2. EIA reactivities of antibody-containing supernatants from hybridoma cell cultures with heat-killed bacterial whole-cell antigens

Antigen preparation	EIA reactivity with the following hybridoma[a]:																										
	P5C9[b,c]	5D10[c,d]	5D10F3[c,d]	5D10C1[c,d]	8A8[c,e]	5B11[f]	3A10[f]	4A2[f]	P3H11[g]	P2D4[g]	P2H7[g]	P4D9[g]	N3D5[h]	N3C8[h]	N3E6[h]	N3A5[h]	N1F2[h]	N2A2[h]	N4G10[h]	P4G2[g]	P2H6[g]	P3A3[g]	P3G8[g]	P1H4[g]	P2H5[g]	P1D12[g]	N1F5[h]
L. monocytogenes																											
Scott A	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	−	+	+	−	−	+	−	−	+	−	+	+
V7	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	−	−	+	−	+	+
Brie-1	+	+	+	+	+	+	+	+	NT[i]	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LCDC 81-861	+	+	+	+	+	+	+	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
ATCC 15313	+	+	+	+	+	+	+	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
ATCC 35152	+	+	+	+	+	+	+	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
L. innocua ATCC 33090	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	+	+	+	+	+	+	+	+	−	+	NT
L. welshimeri ATCC 35897	+	+	+	+	+	+	+	+	+	−	+	−	−	−	+	−	+	−	−	−	+	−	−	+	−	+	NT
L. seeligeri MC 1412	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	−	+	−	+	+	−	+	NT
L grayi ATCC 19120	−	+	+	+	+	+	+	+	+	+	+	−	−	−	+	−	+	V	−	−	+	−	−	+	−	−	NT
L. murrayi ATCC 25401	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	V	+	−	+	+	+	+	NT
L. ivanovii ATCC 19119	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	−	+	+	−	+	NT
B. thermosphacta	−	−	V	V	V	+	+	+	V	V	+	+	V	−	+	+	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	−
C. bovis	−	−	−	−	V	+	+	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
E. rhusiopathiae	−	−	−	−	V	+	+	+	−	−	+	+	−	−	+	+	−	NT	NT	NT	NT	NT	NT	NT	NT	NT	−
L. plantarum	−	NT	NT	NT	+	+	+	+	−	−	−	+	−	−	+	−	V	NT	NT	NT	NT	NT	NT	NT	NT	NT	−
P. cereviseae	−	NT	NT	NT	+	+	+	+	V	−	−	+	−	−	+	−	V	NT	NT	NT	NT	NT	NT	NT	NT	NT	−
S. aureus[j]	−	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	−
S. aureus[k]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	+
S. epidermidis	−	+	−	−	+	+	+	+	V	V	−	+	−	−	+	+	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	−
S. lactis	−	NT	NT	NT	+	+	+	+	V	V	+	+	V	−	+	+	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	−
S. faecalis	−	+	V	V	+	+	+	+	−	+	+	+	−	−	+	+	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	−
S. pyogenes	−	−	−	−	−	+	+	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

[a] +, A_{490} > 2× above background; V, variable between two assays; −, negative. Superscript letters designate fusion myeloma cell lines, immunogens, and adjuvants if used.
[b] P3X, *L. monocytogenes* V7 (method 2).
[c] MAbs from cloned hybridoma cell lines.
[d] P3X, *L. monocytogenes* V7 (method 2), alum precipitated.
[e] NS1, *L. monocytogenes* Scott A (method 1), alum precipitated.
[f] P3X, *L. monocytogenes* Scott A (method 2), poly(A)-poly(U).
[g] P3X, *L. innocua* ATCC 33090 (method 2).
[h] NS1, *L. innocua* ATCC 33090 (method 2).
[i] NT, Not tested.
[j] Supernatant of heated whole-cell extract free of cell wall components.
[k] Heat-treated whole-cell antigen.

conjugate described earlier or alkaline phosphatase-conjugated sheep anti-mouse IgG (Sigma catalog no. A-5781). In the latter case, the substrate used was 5-bromo-4-chloro-3-indolylphosphate–Nitro Blue Tetrazolium.

RESULTS

Reactivities of antibodies against *Listeria* spp. A total of eight separate fusions resulted in 859 viable hybrids. Each hybrid was initially screened for reactivity against *L. monocytogenes* Scott A and V7 antigen preparations. On the basis of screening results, 27 were chosen for further study (Table 2). Of these, MAb P5C9 showed the greatest degree of specificity, reacting with only *L. monocytogenes*, *L. innocua*, and *L. welshimeri* when assayed in the microtiter plate and dot blot EIA formats. None of the other bacteria tested gave positive results with MAb P5C9, except for the nonspecific reaction obtained with whole cells of the protein A-containing strain of *S. aureus*. MAb P5C9 was found to be of the IgG1 murine subclass.

Of the other 26 hybrids, 20 gave positive results with either strain Scott A or V7 antigen and also reacted with all or some of the other six *Listeria* species and non-*Listeria* bacteria. N3C8 reacted only with *L. murrayi*, *L. seeligeri*, and *L. ivanovii* and not with any of the non-*Listeria* test bacteria. Hybrid P2G5 reacted only with *L. murrayi* of the *Listeria* strains, but cross-reactivity with non-*Listeria* strains was not tested. Three of the 20 hybrids, 3A10, 4A2, and 5B11, were positive for all six test strains of *L. monocytogenes* but also reacted with all of the gram-positive non-*Listeria* bacteria tested.

Initially, MAb immunoassays of our cellular antigens using standard microtiter plate assay procedures gave variable results. Further testing of four different brands of polystyrene plates indicated that Immobilon 1 polystyrene microtiter plates (Dynatech Laboratories) gave the best binding of our test antigens under our assay conditions (G. R. Siragusa, M. G. Johnson, and L. N. Raymond, Poultry Sci. **67:**157, 1988). To prove that the lack of MAb reactivity against any particular whole-cell antigen preparation was not due to lack of antigen binding on polystyrene microtiter plates, dot blot assays of these antigens quantitatively immobilized onto nitrocellulose membranes were performed. Dot blot assay results obtained with MAb P5C9 were the same as those obtained with microtiter plate assays under optimal assay conditions (Table 2; Fig. 1). MAb P5C9 showed reactivity only toward strains of *L. monocytogenes*, *L. innocua*, and *L. welshimeri*, and with the antibody-identifying conjugate system used, this MAb detected as few as 10^5 to 10^6 cells of these three strains per ml immobilized on nitrocellulose membranes (Fig. 1). MAb P5C9 did not react with any of the other *Listeria* spp. shown in Table 1, any of the non-*Listeria* spp. listed in Materials and Methods, or eight unidentified gram-positive isolates obtained from *Listeria* selection protocols. MAb P5C9 also reacted with live or Formalin-treated cells of these same three *Listeria* spp. in the dot blot and microtiter plate assay protocols. However, *Listeria* cells that were autoclaved (121°C for 15 min) before assay or fixed to microtiter plates with methylglyoxal (4) did not show reactivity with MAb P5C9. Conversely, the *Listeria* antigens similarly fixed with methylglyoxal in other portions of the same assay plates did react with purified rabbit anti-*Listeria* polyclonal IgG antibody raised against whole *L. monocytogenes* V7 antigen prepared by method 2. These results indicate that the latter two treatments destroyed or masked the antigen recognized by MAb P5C9.

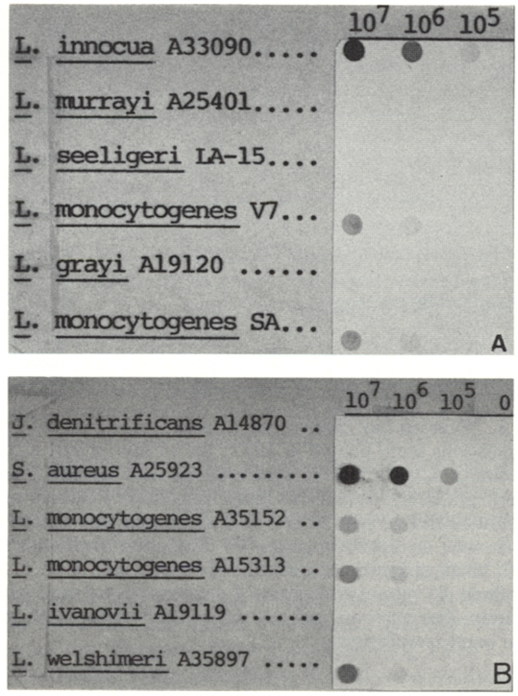

FIG. 1. Dot blots of *Listeria* species (A and B) and *J. denitrificans* (B) immunoprobed with MAb P5C9. Cell suspensions were heated at 80°C for 1 h, blotted at the cell number indicated, and immunoprobed as described in Materials and Methods. *S. aureus* ATCC 25923 (B) is a protein A-containing strain included as a control.

Immunochemical analysis of MAb P5C9 reactivity. For each species, antigens extracted from whole-cell preparations of *L. monocytogenes*, *L. innocua*, or *L. welshimeri*, when analyzed by Western blot analysis and probed with MAb P5C9, yielded a single reactive band with the same migration distance (Fig. 2). The molecular weight of this

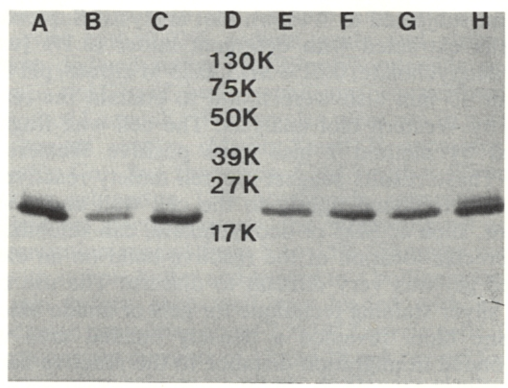

FIG. 2. Western blot analysis of selected *Listeria* strains. Whole-cell suspensions were heated at 100°C for 15 min in PB, mixed 1:1 in Laemmli sample buffer, separated by SDS-PAGE (4% and 12% discontinuous acrylamide), transferred to an Immobilon P membrane, and immunoprobed with MAb P5C9 as described in Materials and Methods. Lanes: A, *L. monocytogenes* V7; B, *L. monocytogenes* Scott A; C, *L. innocua* ATCC 33090; D, molecular weight standards; E, *L. welshimeri* ATCC 35897; F, *L. monocytogenes* ATCC 35152; G, *L. monocytogenes* ATCC 15313; H, *L. innocua* C-194. The numbers indicate molecular weights (10^3).

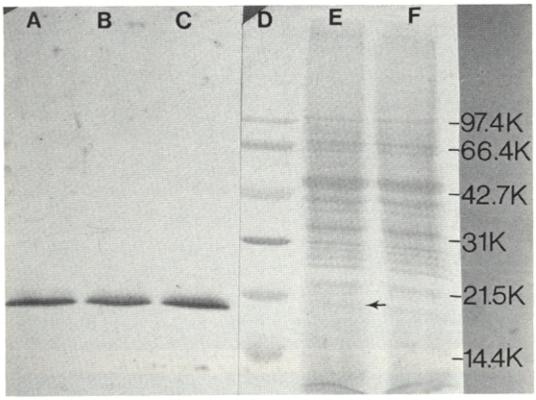

FIG. 3. Western blot analysis of *L. monocytogenes* V7. Whole-cell suspensions were heated at 100°C for 15 min in PB, mixed 1:1 with Laemmli sample buffer, separated by SDS-PAGE (5 to 15% acrylamide gradient), transferred to an Immobilon P membrane, and either immunoprobed with MAb P5C9 or stained with amido black. Lanes: A to C, *L. monocytogenes* V7 (immunoprobed with P5C9); D, amido black-stained molecular weight standards; E to F, amido black-stained *L. monocytogenes* V7 suspension extracts showing total protein. The numbers indicate molecular weights (10^3). Arrow, Reactive band (see Results).

reactive antigen was calculated to be 18,500 (±300) under both reducing and nonreducing conditions. Western blots of the four other recognized *Listeria* species, *L. grayi*, *L. ivanovii*, *L. murrayi*, and *L. seeligeri*, and of *J. denitrificans* gave no reactive bands when probed with this MAb. The reactive polypeptide was stained with the general protein stain Coomassie blue, as well as amido black, and appeared to be partially protein in nature and likely a glycoprotein or lipoprotein moiety.

Western blots of the supernatants from cell suspensions heated in an aqueous detergent-free buffer (PB) were negative, indicating that the reactive epitope was not extractable under these conditions. Pelleted cells from the same cell suspension sample used to obtain the detergent-free PB supernatant did reveal the reactive epitope when suspended in PB and subjected to Western blot analysis. Supernatants from cells extracted with detergent buffer (0.1% [wt/vol] sodium deoxycholate, 0.01% SDS, 0.15 M citrate, pH 8.2) at 100°C for 15 min also were found to contain the reactive antigen by Western blot analysis. The cell wall fragments prepared by method 2 also gave positive Western blot results. These results suggest the cell moiety reactive with MAb P5C9 is bound tightly to the cell wall and a strong detergent, such as SDS or deoxycholate, can solubilize it.

The precise location of the reactive band on an electrophoresed gel was very difficult to discern. The use of the general silver staining technique for gels or amido black for membrane blots revealed a densely banded area which corresponded in migration distance to the reactive band on the corresponding MAb P5C9-immunoprobed membrane blot. The use of a gradient gel helped to resolve the bands better, such that a single silver-stained band (Fig. 3, arrow) lined up with the only band that reacted with the MAb P5C9 in Western blots.

DISCUSSION

Rapid screening of food samples for *Listeria* species is of foremost concern to the food industry. Although *L. monocytogenes* is the main *Listeria* species recognized as a human pathogen (10, 24), under current regulations, the presence of viable cells of any *Listeria* species in cooked, ready-to-eat foods is a cause for concern in the United States. *Listeria* test kits commercially available in the United States react with all *Listeria* species. Subsequent biochemical tests required to identify *L. monocytogenes* can take an additional 3 or more days beyond the 2 days needed for selective enrichment (21).

Rapid assays that could identify *L. monocytogenes* in initial enrichments would save analysis time and would be useful in the study of human listeriosis and its transmission through foods. Therefore, we developed a MAb which shows specificity for three of the seven species of the genus *Listeria*, i.e., *L. monocytogenes*, *L. innocua*, and *L. welshimeri*. The only apparent biochemical difference between the latter two species is the ability of *L. welshimeri* to ferment the sugar xylose (33).

The specificity of MAb P5C9 for 31 strains of *L. monocytogenes* and just the other two above-mentioned *Listeria* species was confirmed (Table 2). Additional assays showed that this MAb did not react with any of the heat-killed whole-cell antigens made from a wide range of other non-*Listeria* gram-positive (22 species) and gram-negative (11 species) bacteria (see Materials and Methods). The dot blot and microtiter plate assay results obtained with MAb P5C9 and whole-cell antigens were the same. Bacteria that were negative in microtiter plate assays were also negative in dot blot assays, indicating that negative results for those bacteria were not the result of poor cell binding to wells of microtiter plates.

Initial P5C9-reactive epitope characterization revealed an 18,500-dalton entity that appeared to be partially proteinaceous. The protein was apparently located in the bacterial cell wall fraction and was extractable with detergent-containing buffers. The molecular weight of the reactive polypeptide was the same in all three positively reacting species, which suggests that it is a common antigen shared among *L. monocytogenes*, *L. innocua*, and *L. welshimeri*. This is the first report of such an antigen of this molecular weight common to these three *Listeria* species and adds to the antigenic characterization of the genus *Listeria* published previously (3, 6, 8, 14, 19, 30, 32, 37, 41; B. Swaminathan, W. E. DeWitt, G. M. Carlone, S. E. Johnson, L. Pine, G. Malcolm, C. Kim, and L. Williams, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, abstr. no. B-255, p. 73).

Delvallez et al. (6) isolated a surface-specific soluble antigen from *L. monocytogenes* serotype 4b (designated antigen 2) which was shown to be present in all of the serovariants of *L. monocytogenes* they tested, as well as in *L. grayi*. Although it was not tested, those workers speculated that *L. murrayi* also contained antigen 2 because of its close taxonomic relationship to *L. grayi*. The relatedness of these two species to the other five *Listeria* species has been debated. An earlier proposal to group these two into a new genus, *Murraya* (39), has since been debated (34) because of similarities the species share. Delvallez et al. (6) also reported the presence of antigen 2 in an isolate identified as *L. monocytogenes* serotype 5, which has since been reclassified (37) as an entirely different species, *L. ivanovii*, a strongly hemolytic species known to be a pathogen of veterinary concern. By using SDS-PAGE under reducing conditions with Laemmli system electrophoresis buffers and parameters similar to those used in our study, Carlier et al. (3) found that antigen 2 had a molecular weight of 16,710 (±450). It is unlikely that the listerial antigen recognized by MAb P5C9 is related to the antigen 2 described by those workers, because the molecular weights of the two antigens differ and antigen 2 was found in two species (*L. grayi* and *L. ivanovii*) with which MAb P5C9 does not react.

The listerial antigen recognized by MAb P5C9 is also apparently different from that reported by Butman et al. (1). Their pan-*Listeria* MAb reacted with an antigen which was common to all seven *Listeria* species and had a molecular weight of 30,000 to 38,000. The wide range of positive reactivities found for our other 26 hybridoma antibodies and MAbs (in supernatants from hybridoma cell culture supernatants) toward *Listeria* species and other genera of gram-positive bacteria is not surprising in light of common antigens shared by *Listeria* species and non-*Listeria* organisms (10, 12, 15, 28, 29, 31, 41).

In other reports of MAbs against *L. monocytogenes*, the immunogens used were either heated whole-cell extracts or soluble protein extracts (1, 5, 6, 25–27, 32, 43; Swaminathan et al., Abstr. Annu. Meet. Microbiol.). The immunogen we used to prepare MAb P5C9 was a partially purified cell wall preparation (method 2) from serotype 1/2a (*L. monocytogenes* V7) and not whole cells or soluble proteins.

McLauchlin et al. (25–27) used wild-type clinical isolates of *L. monocytogenes* as immunogens and produced MAbs reactive with *L. monocytogenes* and either *L. innocua* or *L. seeligeri* when evaluated by a fluorescent-antibody protocol. Ziegler and Orlin (44) reported using serotype 1 *L. monocytogenes* antigen to produce MAbs which had genus and species specificity when evaluated by radioimmunoassay. However, the latter workers did not report screening their MAbs for reactivity against antigens of *L. innocua*, *L. welshimeri*, or *L. seeligeri*.

In summary, the P5C9 MAb described here has the potential for use in the development of more specific *Listeria* immunoassays for food, environmental, and clinical specimens, such as colony immunoblot tests, as well as in epidemiological studies or as a more rapid identification reagent for these species for use in conjunction with existing isolation protocols. Of the other 26 hybridomas generated, 20 secreted antibodies which reacted with some strains of *L. monocytogenes* but also cross-reacted with some non-*Listeria* bacteria. The MAb P5C9-reactive polypeptide appears to be a cell-bound protein that can be extracted by detergent treatment, has a molecular weight of 18,500 (±300, under both reducing and nonreducing conditions), and is found in *L. monocytogenes*, *L. innocua*, and *L. welshimeri* but not in the other four species of the genus *Listeria*.

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